

## Hereditary Nephrogenic Diabetes Insipidus: Molecular Basis of the Defect and Potential Novel Strategies for Treatment

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### Abstract

The antidiuretic hormone vasopressin regulates water reabsorption in the nephron by inducing apical plasma membrane exocytosis of the water channel aquaporin 2 in the kidney collecting duct principal cells. Disruption of this physiological mechanism by genetic alteration of either the vasopressin type 2 receptor gene or the aquaporin 2 gene, results in a rare genetic disorder known as nephrogenic diabetes insipidus, which main hallmark are polyuria and polydipsia. Over the last decades, analysis of patients affected by this disease helped genetists, clinicians, cell and molecular biologists and pharmacologists to better understand the physiology of water reabsorption in the kidney, the molecular basis of the disease and to propose protocols for rapid diagnosis and pharmacological handling of the disease.

Much still remains to be done in terms of targeted therapy to make sure that these patients benefit from an improved quality of life. In this article we provide an overview on the most recent strategies under investigation for rescuing the mutated gene products activity or for bypassing defective vasopressin receptor signaling.

**Keywords:** Nephrogenic diabetes insipidus; Vasopressin; AVPR2; Aquaporin 2; Polyuria; CAMP; Statins

### Introduction

Water inside and outside the cells represent approximately 60% of total human body weight. The maintenance of fluids homeostasis is essential for most physiological processes. For this reason, urinary excretion of water is finely regulated to allow a rapid adaptation to water uptakes and losses and maintain constant salt concentration in intra- and extracellular fluids.

Glomerular filtration produces up to 180 l/day of pro-urine within the Bowman's capsule although, due to the massive water reabsorption in the renal tubule, only less than 1% of the initial volume will be excreted. Indeed, in the proximal tubule and in the Henle's loop, about 90% of the water is subject to obligatory reabsorption through the water channel aquaporin 1 (AQP1) [1]. The remaining 18-20 L of urine reaching the distal tubule and the collecting duct are subjected to regulated water reabsorption according to plasma osmolality and blood volume.

Increases in plasma osmolality (hypernatremia) or decreases of the blood volume (hypovolemia) cause kidneys to conserve water, a physiological condition known as antidiuresis. In particular, stimulation of hypothalamic osmoreceptors or aortic and carotid baroreceptors triggers the release of the antidiuretic hormone arginine vasopressin (AVP) from the pituitary gland into the bloodstream [2,3]. The antidiuretic action of vasopressin occurs upon binding to type 2 vasopressin receptor (AVPR2) a class of G protein-coupled receptors localized on the basolateral plasma membrane of the principal cell of the kidney collecting duct (Figure 1A). Once activated by AVP, AVPR2 interacts with the Gas, which, in turn, activates adenylate cyclase, increases intracellular cAMP levels and triggers a cascade of intracellular signals mostly mediated by PKA activation. A crucial step in this process is RhoA inhibition and partial depolymerization of subapical actin cytoskeleton [4]. As a final result, a pool of intracellular storage vesicles containing the water channel aquaporin 2 (AQP2) fuses with the apical membrane of the principal cells making the plasma membrane highly water permeable at this site. The osmotic

gradient due to solute reabsorption in the medullary thick ascending limb (TAL), also regulated by AVP, provides the driving force for AQP2-mediated water reabsorption within the principal cells. The exit pathway for water entering the cells is represented by aquaporin 3 and 4 (AQP3/4) localized at the basolateral membrane of the same cells mediating water flux to the extracellular fluid and ultimately to blood. This process restores plasma osmolality and volume and is regulated by negative feedback. In fact, upon restoration of water balance, the levels of plasma AVP drop, AQP2 levels in the apical plasma membrane decrease by endocytosis and less water is reabsorbed in the collecting duct (diuresis). During antidiuresis AVP increases urine osmolality up to 1200 mos m/kg and decrease urine flow to 0.5 ml/min. On the other hand, AVP removal from the blood stream or AVPR2 desensitization/internalization [5] elicits diuresis, characterized by low urine osmolality (below 250 mosm/kg) and higher urine output (around 2 ml/min) [6].

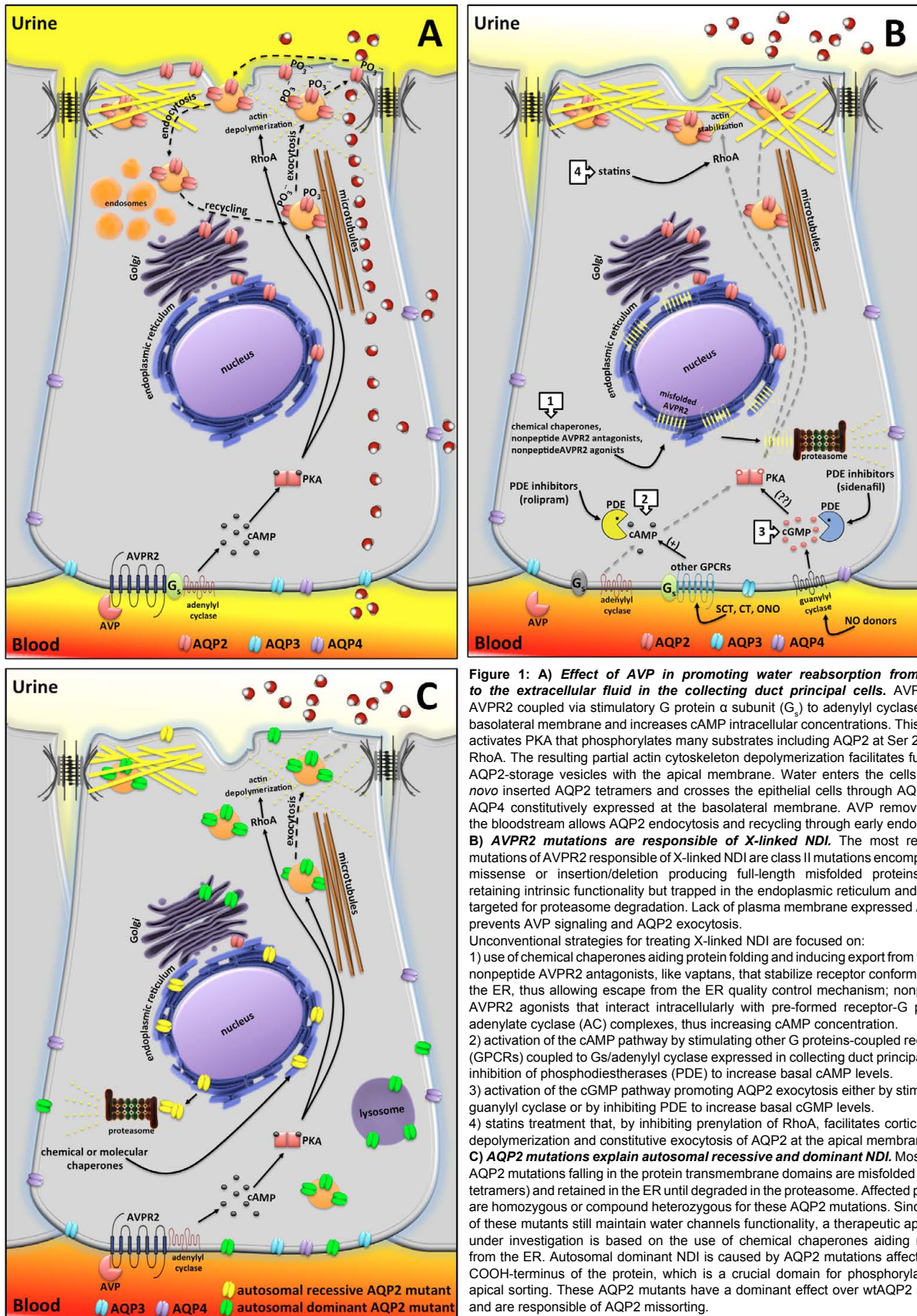
It can be easily understood that alterations in the production/release of vasopressin, binding to its cognate AVPR2 receptor and in the trafficking of AQP2 result in severe impairment of water reabsorption in the kidney. The congenital form of nephrogenic diabetes insipidus (NDI) is a rare inherited disorder, characterized by insensitivity of the distal nephron to the antidiuretic effects of AVP. As a consequence, the kidney loses its ability to concentrate urine, which may lead to severe dehydration and electrolyte imbalance (hypernatremia and hyperchloremia).

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**Figure 1: A) Effect of AVP in promoting water reabsorption from urine to the extracellular fluid in the collecting duct principal cells.** AVP binds AVPR2 coupled via stimulatory G protein  $\alpha$  subunit ( $G_s$ ) to adenylyl cyclase at the basolateral membrane and increases cAMP intracellular concentrations. This in turn activates PKA that phosphorylates many substrates including AQP2 at Ser 256 and RhoA. The resulting partial actin cytoskeleton depolymerization facilitates fusion of AQP2-storage vesicles with the apical membrane. Water enters the cells *via de novo* inserted AQP2 tetramers and crosses the epithelial cells through AQP3 and AQP4 constitutively expressed at the basolateral membrane. AVP removal from the bloodstream allows AQP2 endocytosis and recycling through early endosomes.

**B) AVPR2 mutations are responsible of X-linked NDI.** The most recurrent mutations of AVPR2 responsible of X-linked NDI are class II mutations encompassing missense or insertion/deletion producing full-length misfolded proteins often retaining intrinsic functionality but trapped in the endoplasmic reticulum and mostly targeted for proteasome degradation. Lack of plasma membrane expressed AVPR2 prevents AVP signaling and AQP2 exocytosis.

Unconventional strategies for treating X-linked NDI are focused on:

- 1) use of chemical chaperones aiding protein folding and inducing export from the ER; nonpeptide AVPR2 antagonists, like vaptans, that stabilize receptor conformation in the ER, thus allowing escape from the ER quality control mechanism; nonpeptide AVPR2 agonists that interact intracellularly with pre-formed receptor-G protein-adenylyl cyclase (AC) complexes, thus increasing cAMP concentration.
- 2) activation of the cAMP pathway by stimulating other G proteins-coupled receptors (GPCRs) coupled to Gs/adenylyl cyclase expressed in collecting duct principal cells; inhibition of phosphodiesterases (PDE) to increase basal cAMP levels.
- 3) activation of the cGMP pathway promoting AQP2 exocytosis either by stimulating guanylyl cyclase or by inhibiting PDE to increase basal cAMP levels.
- 4) statins treatment that, by inhibiting prenylation of RhoA, facilitates cortical actin depolymerization and constitutive exocytosis of AQP2 at the apical membrane.

**C) AQP2 mutations explain autosomal recessive and dominant NDI.** Most of the AQP2 mutations falling in the protein transmembrane domains are misfolded (yellow tetramers) and retained in the ER until degraded in the proteasome. Affected patients are homozygous or compound heterozygous for these AQP2 mutations. Since most of these mutants still maintain water channels functionality, a therapeutic approach under investigation is based on the use of chemical chaperones aiding release from the ER. Autosomal dominant NDI is caused by AQP2 mutations affecting the COOH-terminus of the protein, which is a crucial domain for phosphorylation or apical sorting. These AQP2 mutants have a dominant effect over wtAQP2 subunit and are responsible of AQP2 missorting.

See text for more detailed explanation.

Frequently, NDI can be an acquired pathological condition resulting as side effect of pharmacological treatments with lithium [7], drugs [8], antibiotics/antifungal/antiviral [9-14]. Hypokalemia or hypercalcemia/hypercalciuria can also cause acquired NDI [15-19] as well as acute and chronic renal failure (ARF, CRF) [20-25]. In most cases downregulation of AQP2 expression or altered trafficking are responsible for acquired NDI. In this review we will focus our attention on the genetic defects leading to congenital NDI.

## Diagnosis of NDI

NDI causes polyuria (i.e. a urine output exceeding 3 L/day in adults and 2 L/m<sup>2</sup> in children) in which large amounts of dilute urine (i.e., urine osmolality usually below 250 mosmol/kg) are excreted. Other causes of polyuria, apart from glucose-induced osmotic polyuria due to uncontrolled diabetes mellitus, are primary (psychogenic polydipsia), and central (neurohypophyseal or neurogenic) DI. History, measurement of urine osmolality, and plasma sodium concentration are essential part of the initial diagnostic workup of NDI.

## Clinical history

Polyuria appearing in the first week of life suggests hereditary NDI, while polyuria starting during the first year of life or in young adulthood suggests a familial central DI [26]. Sudden polyuria in adulthood suggests central DI, while a gradual onset usually occurs in acquired NDI (chronic lithium use and hypercalcemia) or primary polydipsia. A family history of polyuria is typical of hereditary NDI and familial forms of central DI.

## Measuring plasma sodium, urine osmolality, and urine output

Features based on measurement of urine osmolality and plasma sodium concentration may distinguish between NDI, primary polydipsia, and central DI, all causing polyuria and dilute urine output. NDI and central DI have high-normal plasma sodium concentration (>142 mEq/L) and urine osmolality lower than plasma osmolality, while primary polydipsia is characterized by low urine osmolality and low plasma sodium concentration (below 137 mEq/L). Hypernatremia (i.e., plasma sodium concentration above 150 mEq/L) is unusual in adults with DI and without cognitive impairment or central lesions, because water loss stimulates thirst, which leads to increased water intake. In children with hereditary NDI, severe thirst might appear during the first year of life, can be underestimated and might cover up a condition of hypernatremia. These children with hereditary NDI and no family history of NDI might represent the first affected family member in whom a *de novo* mutation occurs or an X-linked mutation takes place in asymptomatic females [27]. If polyuria is abundant, urine output should be performed every 8 hrs rather than every 24 hours. Measurement of urinary creatinine excretion confirms that collection is complete.

## Water restriction test (WRT)

The initial suspicion of NDI must be confirmed by a test which will increase plasma osmolality; a WRT or administration of hypertonic saline (in adults: 0.05 mL/kg per min up to 120 min if water restriction test is inconclusive) are usually performed [28]. In healthy subjects, raising plasma osmolality correlates with progressive increase in AVP release and urine osmolality, with maximal AVP effect on the kidney for a plasma osmolality around 300 mosmol/Kg or a plasma sodium  $\geq$  145 mEq/L. Desmopressin administration (10  $\mu$ g by nasal insufflation or 4  $\mu$ g s.c. or i.v.) will not increase urine osmolality further (unless

central DI and impaired AVP release are present). In adults, the WRT is typically performed under close medical supervision, and by interrupting water ingestion for 2-3 hrs while urine osmolality and volume are performed every hour, and plasma osmolality and the sodium concentration are performed every two hours. The WRT is ended in the presence of one of the following results, i.e., when urine osmolality raises to above 600 mosmol/Kg (normal status, AVP release and function; lower values are suggestive of DI); when urine osmolality remains stable despite a rising plasma osmolality in the following 2 to 3 hrs; plasma osmolality is higher than 295 to 300 mosmol/kg or plasma sodium is equal or higher than 145 mEq/L. Desmopressin is administered in the last two cases while monitoring urine osmolality and volume (every 30 minutes over the next 2 hours). If the response to water restriction test is inconclusive, measurement of plasma and urine AVP levels are performed. In newborns or young infants the suspicion of hereditary NDI (e.g., a plasma sodium equal to or greater than 145 mEq/L plus a urine osmolality equal or less than 200 mosmol/kg) is confirmed by administration of desmopressin (1  $\mu$ g s.c. or i.v. over 20 minutes, maximum dose 0.4  $\mu$ g/kg of body weight) together with measurement of urine osmolality (baseline and every 30 minutes over the next 2 hours), without WRT. NDI is diagnosed if the increase of urine osmolality is less than 100 mosmol/kg over baseline. Genetic analysis is advisable in these cases [26]. Different patterns can result from water restriction test and desmopressin administration [28,29]. In NDI, submaximal increase of urine osmolality occurs on WRT. With desmopressin, neither rise in urine osmolality (in complete NDI) nor up to 45% rise in urine osmolality (in partial NDI) are observed. In central DI, submaximal increase of ADH release, urine osmolality and plasma osmolality are observed. Desmopressin is associated with supra-maximal or partial (15-50%) increase of urine osmolality (in complete or partial central DI, respectively). WRT might lead to inconsistent results in some cases of partial central DI or during pregnancy (vasopressinase effect) [28,30]. In patients with partial NDI a mild increase (< 600 mOsm/Kg) in urinary osmolality is achieved after desmopressin infusion [31].

## Measuring AVP in plasma and urine

In uncertain cases, AVP is measured in plasma and urine at baseline and during water deprivation (before giving AVP) [29,32]. NDI is excluded if urine osmolality rises with increased AVP secretion. Limitations of the test are due to low sensitivity of commercially available assays for measurement of plasma and urinary AVP, binding of AVP to circulating platelets (falsely high and low levels), and instability of plasma AVP.

## Measuring copeptin in plasma

The role of the copeptin test in the diagnosis of DI, albeit promising is still experimental [33]. Copeptin is a glycopeptide (39-amino acid) comprising the C-terminal part of the AVP precursor (CT-proAVP). Copeptin acts as a stable and sensitive surrogate marker for AVP release. Plasma copeptin levels are measured by immunoassay during the water deprivation test. In a recent study, plasma levels of copeptin greater than 20 pmol/liter identified patients with NDI, while concentrations below 2.6 pmol/liter indicated complete central DI [34].

## Other differential diagnoses

NDI produces water diuresis, meaning dilute urine and excretion of normal total solute (600-900 mosmol/day). Other conditions need to be differentiated and should consider the characteristics of solute diuresis (urine osmolality > 300 mosmol/kg). Glycosuria usually occurs

in patients with poorly controlled diabetes mellitus, or with severe central DI and AVP resistance undergoing infusion of large volumes of dextrose and water. Urea-dependent polyuria is also observed after resolution from azotemia, therapy with urea in patients during hyponatremia, tissue catabolism, and high amounts of proteins given orally or intravenously. Sodium diuresis with polyuria may result from administration of large volumes of intravenous saline or in patients with bilateral urinary tract obstruction [35-37].

### Genetic analysis

When history, measurement of urine osmolality and plasma sodium concentration suggests the hereditary NDI, genetic testing should be performed. Families with hereditary DI and asymptomatic members of affected families should be considered for molecular defects. In newborns or young infants with suspicion of hereditary NDI, and a positive desmopressin test, genetic analysis is also indicated [26]. Most frequent conditions include mutations in the AVPR2 gene encoding the V2 receptor or mutations in the aquaporin-2 (water channel gene) [26].

The risks to sibs and offspring depend on the mode of inheritance and the carrier status of the parents. Prenatal testing is possible for at-risk pregnancies if the disease-causing mutation(s) in the family have been identified. Molecular genetic testing used in NDI includes methods like sequence analysis, deletion/duplication analysis, and linkage analysis. Mutations detected include sequence variants, exonic and whole-gene deletions/duplications, and sequence variants [38].

### Pathophysiology of Nephrogenic Diabetes Insipidus

NDI is a syndrome which clinical hallmarks are polyuria and compensatory polydipsia. Upon water restriction or inadequate water supply, patients suffering from NDI do not properly compensate water loss and are at risk of severe dehydration. Congenital NDI is caused by mutations in the AVPR2 or the AQP2 genes, respectively. As a consequence the distal nephron is insensitive to AVP resulting in blunted water reabsorption in the collecting duct. Three different inheritance patterns of NDI have been recognized. In most cases (about 90%), NDI is transmitted as an X-linked recessive trait (MIM #304800) caused by mutations in the AVPR2 receptor gene located on the X chromosome [39]. A minority of patients (about 10%) show an autosomal recessive (MIM #222000) or dominant trait (MIM #125800) as a result of mutations in the AQP2 gene located on chromosome 12q13 [40]. The urine concentrating defect is present at birth and symptoms arise during the first week of life as irritability, poor feeding, failure to thrive and seizures [41]. Signs of dehydration are dryness of the skin, loss of normal skin turgor, recessed eyeballs, increased periorbital folding, and depressed anterior fontanel. Upon initiation of pharmacological treatment (see below) most recover their weight loss [42]. The persistent polyuria can cause development of kidney megacystis, trabeculated bladder, hydroureter, and hydronephrosis [41].

Repeated episodes of dehydration followed by too fast rehydration, can cause brain edema that leads to mental retardation [43,44], a serious complication of NDI [45]. Nowadays this complication is rare due to earlier recognition and treatment of NDI. Nevertheless, psychological development of these patients is adversely affected by the persistent need for drinking and frequent voiding.

### Defects in the AVPR2 gene lead to X-linked NDI

The AVPR2 gene was first described in 1992 [46] and is a typical

seven membrane-spanning helices G protein-coupled receptor (GPCR).

Mutations in the AVPR2 gene lead to X-linked NDI (X-NDI) [47]. This is the cause of 90% of all diagnosed congenital NDI cases.

While affected male patients do not concentrate urine even after administration of exogenous AVP [48], due to skewed X-chromosome inactivation, some heterozygous females have variable degrees of polyuria and polydipsia [27,49,50]. Depending on the position of the mutation, partial or incomplete phenotype can be seen in some patients [51]. The number of AVPR2 mutation leading to X-NDI is constantly increasing. According to the Human Gene Mutation database (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=AVPR2>) 247 out of 249 identified mutations of AVPR2 gene are “loss of function” mutations (Table 1). AVPR2 mutations have been assigned to five different classes according to sequence analysis and their subcellular localization [52]. Class I mutation interfere with proper transcription, mRNA processing and translation, leading to truncated proteins [53]. The truncated mutants are then rapidly degraded, and therefore cannot be expressed at the cell membrane.

Class II mutations, the most common, are missense or insertion/deletion producing full-length misfolded proteins retained in the endoplasmic reticulum (ER) and mostly targeted for proteasome degradation [54] (Figure1B). Class III mutants result in plasma membrane-expressed receptors with reduced affinity for the stimulatory Gs protein, leading to blunted activation of the phosphorylation pathway promoting AQP2 exocytosis [53]. Class IV mutants have low affinity for vasopressin [53]. Class V mutants are misrouted to different subcellular organelles [55,56].

In addition, the AVPR2 can also be affected by “gain of function” mutations. These mutations cause increased binding affinity to

Mutation type	Chromosome location	Phenotype	N° of mutations
<b>AQP2</b>			
Missense/nonsense	12q12-q13	Autosomal recessive NDI	40
	12q12-q13	Autosomal dominant NDI	3
Splicing	12q12-q13	Autosomal recessive NDI	3
Small deletions	12q12-q13	Autosomal recessive NDI	5
	12q12-q13	Autosomal Dominant NDI	4
Small insertions	12q12-q13	Autosomal Dominant NDI	1
<b>TOTAL</b>			<b>56</b>
<b>AVPR2</b>			
Missense/nonsense	Xq28	X-linked NDI	151
Splicing	Xq28	X-linked NDI	3
Small deletions	Xq28	X-linked NDI	47
Small insertions	Xq28	X-linked NDI	14
Small indels	Xq28	X-linked NDI	5
Gross deletions	Xq28	X-linked NDI	22
Gross insertions	Xq28	X-linked NDI	1
Complex rearrangements	Xq28	X-linked NDI	4
<b>TOTAL</b>			<b>247</b>
<b>L1CAM</b>			
Gross deletions	Xq28	Hydrocephalus & X-linked NDI	1
Complex rearrangements	Xq28	Hydrocephalus & X-linked NDI	1
<b>TOTAL</b>			<b>2</b>

**Table 1:** Overview and classification of mutations causing nephrogenic diabetes insipidus (NDI) as reported by HGMD® Professional 2013.4 as of December 2013.

AVP [57] or constitutive activation of the receptor, resulting in the nephrogenic syndrome of inappropriate antidiuresis (NSIAD) [58-60].

In addition to mutations in the AVPR2, gross gene deletions or complex rearrangement of the L1CAM gene, mapping adjacent to the AVPR2 [61], can result in NDI [62-64] (Table 1). However, only L1CAM gene deletions that also encompass the AVPR2 are associated with NDI, while isolated point mutations in the L1CAM gene are often associated with hydrocephalus [65].

### Defects in AQP2 gene lead to autosomal recessive/dominant NDI

The AQP2 gene is located on chromosome 12q13. The gene codes for the 271 amino acid AQP2 protein, which consists of six transmembrane domains connected by five loops and intracellularly located N- and C-termini (type IV-A TM protein) [66]. Tetramerization of monomers takes place in the ER [67]. High-mannose glycans are attached to one or two monomers of a homotetramer in the ER and further processed to complex glycans in the trans Golgi network [68]. Glycosylation of AQP2 is not required for passing ER quality control and ER export, but is essential for post-Golgi trafficking in mammalian cells [67].

Phosphorylation of serine 256 is required for cAMP-dependent regulatory exocytosis of the aquaporin-2 water channel [69]. In 2006, large-scale phospho-proteomic analysis demonstrated that this S256 phosphorylation site is part of a polyphosphorylated region in the COOH-terminal tail of AQP2 encompassing S261, S264, and S269 [70]. The use of mutated AQP2 expressed in polarized kidney epithelial cell lines suggests that polyphosphorylation of AQP2 COOH-terminal occurs as a hierarchical event, with S256 phosphorylation being required for phosphorylation of S264 and S269 [71]. Studies *in vitro* and *in vivo* strongly suggest a role for both S256 and S269 in membrane accumulation of AQP2 [71-73]. With respect to other phosphorylation sites, recent studies suggest that these sites play minimal roles in AQP2 plasma membrane targeting [74,75].

Approximately 10% of NDI patients have the autosomal form of NDI. Similar to AVPR2 inactivating mutations, autosomal alterations in AQP2 disrupt proper synthesis, functioning or localization of the gene product, rendering renal collecting duct principal cells irresponsive to AVP stimulation [48].

Currently, 56 mutations of AQP2 gene have been described as causative of autosomal NDI, most of which show a recessive inheritance (Table 1). Patients are homozygous or compound heterozygous for these AQP2 mutations. Mutations, mostly missense, have been described to fall in the protein transmembrane domains and cause AQP2 to be folded aberrantly. ER accumulation has been demonstrated by several studies [76-80] (Figure 1C). As for AVPR2, ER retention due to extended interaction times with ER chaperones eventually leads to proteasome degradation. However, some mutants retain intrinsic functionality, and show at least partial activity when expressed in the apical membrane by means of forced transport or overexpression [79].

In fact, for most missense mutants, partial water permeability has been shown by ectopic expression in *Xenopus* oocytes [81] indicating that the native conformation is disturbed only slightly. This suggests that the observed disease phenotype is generally due to aberrant subcellular localization of AQP2 rather than loss of function. This is of great therapeutic significance for restoring mutant trafficking.

A small number of AQP2 mutations are inherited in a dominant trait and are causative of autosomal dominant NDI [82-85]. Mutations

affect the carboxyl-terminal of AQP2 containing regulatory sequences for trafficking and sorting. The heterotetramers formed by WT and mutated AQP2 monomers are either retained to the Golgi apparatus [85,86] or misrouted to late endosomes, lysosomes [79] or basolateral membrane [87] (Figure 1C). In some cases residual trafficking to the apical membrane can be detected. This is supposedly due to the fact that one-sixteenth of all tetramers formed in dominant NDI are wt-AQP2-only tetramers [88,89].

### Partial NDI

Several reports have shown that a number of mutations cause a mild form of NDI. The majority of patients with X-NDI display little or no rise in urine osmolality in response to fluid deprivation test or large doses of AVP or desmopressin (dDAVP) (see above, Diagnosis of NDI). Nevertheless, a few patients have been reported to concentrate their urine quite efficiently while subjected to fluid deprivation test, AVP and dDAVP [90,91]. This residual urine concentrating ability does not prevent the symptoms of NDI under basal conditions, but it may protect against the episodes of severe hypertonic dehydration to which patients with severe defects are susceptible. The age of onset of the disease in individuals with X-linked partial NDI due to an AVPR2 mutation usually appears later in life. To date, only 14 of all known missense mutations identified in the AVPR2 gene have been associated with partial X-linked NDI phenotype [91]. Beside genetic defects partial NDI may be also attributable to aging. It has been reported that in both humans and rats aging results in reduced maximal urine concentrating ability because of downregulation of AQP2 and urea transporters [92,93].

### Animal Models for Studying NDI

Until the recent and large-scale development of genetic manipulation technology, which has led to the generation of transgenic mice models, our knowledge on renal AQP2 regulation was mainly based on *in vitro* studies on suitable renal cell models. Transgenic and knockout technology approaches are providing pivotal information on the role of AQP2 and AVPR2 in controlling water homeostasis in health and disease [94]. A variety of AQP2 KO/knock-in mice models of NDI have demonstrated the critical role of AQP2 in maintaining water balance [95]. It must also be mentioned that deletion or mutation of several other genes can result in severe defects in the ability to concentrate urine and resistance of the kidney to AVP, suggesting an "NDI-like phenotype" [96-98]. Transgenic mouse models for NDI are useful to elucidate potential compensatory or adaptive changes in the kidney and to examine targeted therapeutic strategies for specific AQP2 and AVPR2 mutations.

### Autosomal recessive NDI

The recent generation of transgenic mice clearly improved the state of the art regarding the role of AQP2 in controlling water homeostasis in health and disease.

Several models for autosomal recessive NDI have been established, all with poor viability, suggesting that the mice are sensitive to the polyuria [99]. Total AQP2 KO mice died within 2 weeks of age [100] and kidneys from these mice showed papillary atrophy and increase in pelvic spaces, i.e., signs of hydronephrosis development. In contrast, mutant mice expressing AQP2 exclusively in the connecting tubule (CNT) and deficient in the collecting duct (AQP2-CDKO) are viable and reached adulthood but have a severe urinary concentration defect [100]. These findings suggest that the collecting duct is fundamental to the rescue of the lethal phenotype observed in total AQP2 knockout mice

and that it cannot be compensated for by other mechanisms. AQP2-CD-KO mice showed a tenfold increase in urine output compared with control siblings, and water restriction caused only a slight decrease in urine output and no change in urine osmolality, revealing the absence of compensatory mechanisms. These results indicate that AQR2 plays a fundamental role in the collecting duct system in the regulation of osmotic equilibration, although the role of AQP2 in the CNT is still not completely clear.

Similarly to mice totally lacking AQP2, the knock-in mouse model carrying the recessive NDI T126M mutation in homozygous appeared normal at 2-3 days after birth but failed to thrive and generally died by day 6 if not given supplemental fluid [101].

Recently, an inducible knock-in mouse model of NDI was developed and used for evaluating chemical strategies to rescue mutated AQP2 in adult mice [99].

McDill et al. [102] identified a mutation in the AQP2 gene responsible for a severe form of recessive NDI. Interestingly, the described mutation causes the substitution of serine 256 (S256) by a leucine, thus preventing AQP2 phosphorylation at S256 by PKA. The S256L is a spontaneous recessive mutation that causes severe hydronephrosis and obstructive nephropathy in affected mice. No such mutation has been found as yet in humans; however, quite interestingly, the AQP2-R254L mutation, found in humans, also occurs at the PKA consensus site and when expressed *in vitro* also prevents S256 phosphorylation, thus explaining the NDI phenotype [103].

Furthermore, a mouse model with a F204V mutation resulting in recessive NDI supports the hypothesis that defective targeting of AQP2 is the basis for some forms of NDI [77]. F204V mouse model was able to survive to adulthood. Similarly to other missense recessive mutations, AQP2-F204V is located in the core of AQP2 and is mainly retained in the ER. F204V mice more exactly recapitulated the human disorder. The smaller response to dDAVP indicates some residual activity of the mutant AQP2 channel, which must be sufficient to allow survival of the individual, in contrast to the T126M knock-in mouse [101].

In heterozygous mice, Lloyd et al. additionally showed that AQP2-F204V can homotetramerize as well as form heterotetramers with wt-AQP2 [77]. Measurement of the water osmotic coefficient (Pf) using *Xenopus* oocytes demonstrated that AQP2-F204V maintains a residual function as a water channel and that a small amount of mutants escape the ER quality control and is targeted to the plasma membrane, explaining this milder non-lethal phenotype. Therefore, these findings suggest that the severity of a recessive NDI phenotype depends on the degree of AQP2 misfolding, and extend to the possibility of this mutant overcoming ER quality control. Mouse models of NDI have provided direct genetic evidence that specific mutations in AQP2 impair the apical accumulation of the water channel and revealed the genetic basis of the urinary-concentrating defect [94].

### Autosomal dominant NDI

Frame-shift mutations within the C terminus of AQP2 cause autosomal-dominant nephrogenic diabetes insipidus that is the least prominent form of NDI and is responsible for <1% of NDI cases.

To identify *in vivo* the molecular mechanism(s) of this disease and to test possible therapeutic strategies, Sohara generated a mutant AQP2 (763-772 del) knock-in mouse [83]. Similarly to the milder phenotype described in humans, heterozygous mice exhibit an impairment in urine concentrating ability, as well as a slight but significant increase in urine osmolality after fluid deprivation. Immunofluorescence studies

of dehydrated animals revealed that AQP2 was mainly missorted to the basolateral membrane, although a weak staining of wild-type AQP2 was also detected at the apical plasma membrane, thus explaining the milder phenotype of these mice in terms of urinary-concentrating ability [83].

### X-linked NDI

AVPR2-deficient male mice, with a mutation resulting in the premature insertion of a stop codon (E242X), known to cause defective AVPR2 function and X-NDI [104], are polyuric at birth. These pups exhibited an enlargement of renal pelvic space, failed to thrive, and died within the first week after birth due to hypernatremia and dehydration caused by the inability of these animals to concentrate their urine.

The expression of several genes involved in sodium and water reabsorption was upregulated in the kidneys of 3-day-old male AVPR2-deficient mice including AQP1, carbonic anhydrases, the Na<sup>+</sup>-K<sup>+</sup>-ATPase, and NaCl and HCO<sub>3</sub><sup>-</sup> transporters and cyclooxygenase 2 expression, in both the kidney and hypothalamus suggesting compensatory changes [105]. Despite these compensatory changes, E242X mice die within 1 week, making them an unsuitable model for studying X-NDI in adult mice.

Recently, the first viable mouse model of X-NDI has been generated in which the AVPR2 gene can be conditionally deleted during adulthood by administration of tamoxifen [106].

After tamoxifen, adult mice excrete a large amount of hypotonic urine, show changes in renal morphology and lack of response to AVP and have reduced levels of AQP2 and AQP3 [106]. Therefore upon AVPR2 deletion, adult mice displayed all characteristic symptoms of X-NDI: polyuria, polydipsia, and low urine osmolality. The availability of such a valid animal model will help to develop a specific and effective pharmacological therapy for the treatment of NDI.

### Standard Treatment of NDI

One important goal of NDI treatment is to lower urine output in NDI patients. This goal implies a low salt, low protein diet, use of diuretics, and nonsteroidal anti-inflammatory drugs (NSAIDs). Early recognition and therapy are key steps in infants, to avoid recurrent episodes of dehydration and hypernatremia, with consequent mental and physical retardation. In adults, by contrast, the thirst mechanism is usually sufficient to keep plasma sodium levels within the high-normal range. In this case the decision to start treatment depends on a case-by-case condition and tolerance to polydipsia and polyuria.

### General recommendations

Infants do not respond properly to increased thirst. The following steps are therefore advisable in this group of young patients: water offered at 2-hr intervals day and night, continuous gastric feeding in the most severe cases. Complications such as gastroesophageal reflux, abnormal growth and appetite, hydronephrosis, dilated bladder, hydronephrosis, and progressive renal failure, require careful monitoring. Young patients must be instructed to “double voiding” in order to empty the bladder completely, and avoid progressive dilatation of the urinary tract. If i.v. fluids are required in NDI (especially in young patients), much attention must be given to avoid solutions leading to hypernatremia (hypertonic solutions), as well as to hyponatremia (hypotonic solutions).

### Low salt, low protein diet

This approach is valid to reduce urine output in NDI [41]. As

in NDI the urine osmolality is fixed, the variable determining urine output will depend on solute excretion. There is a direct relation between decreased solute intake (sodium, proteins) and urinary excretion [41]. Ideally, sodium intake is lowered to less than 2.3 g/day (i.e. less than 100 mEq/day), while protein intake can be 1.0 g/Kg or less. Poor compliance and the potential dietary harmful effects of a very low protein intake are major concerns.

### Diuretics

Thiazide diuretics effectively reduce polyuria especially if combined with a low solute diet. Urine output could be reduced by almost 70% when hydrochlorothiazide was associated with very low sodium-restricted diet of 9 mEq/day [107]. Hydrochlorothiazide is given as 25 mg daily or b.i.d. [107,108]. Potassium sparing agents such as amiloride, might have an additive effect with thiazide diuretics, via a mechanisms likely including the inhibition of potassium loss induced by thiazides [109]. This effect is clearly visible in patients in lithium-induced NDI [7]. Diuretics in NDI are likely to reduce urine output by promoting proximal reabsorption of sodium and water. In this condition, less water is delivered to the AVP-sensitive tract of the nephron, the collecting duct.

### Nonsteroidal anti-inflammatory drugs (NSAIDs)

Renal prostaglandin synthesis (mediated by the prostaglandin synthetase) is inhibited by NSAIDs. The effect of NSAIDs in NDI is based on the inhibition of the antagonizing effect of prostaglandins on AVP. A better urinary concentration is achieved with NSAIDs, and output in NDI can be reduced by 25-50% [108,110]. Hydrochlorothiazide has additive effects [108,109]. Ibuprofen (25 mg/kg/day) and indomethacin (2 mg/kg/day) were tested in patients aged 8 to 18 years with hereditary NDI [110]. Only indomethacin use produced a consistent decrease of urine volume and free water clearance. Either antidiuretic hormone (AVP)-stimulated cyclic adenosine monophosphate generation or AVP-independent water reabsorption are mechanisms likely to be involved upon NSAIDs administration.

### Exogenous AVP

Exogenous administration of the synthetic AVP analogue desmopressin (dDAVP) was effective in central DI [111] and might be effective in non-hereditary NDI where a partial rather than a complete resistance to AVP exists. The effect is even stronger if dDAVP was combined with NSAIDs [112]. Urine osmolality might increase to more than 40% while urinary output should decrease accordingly, upon exogenous administration of AVP.

In summary, the following steps must be undertaken when managing NDI patients:

- start with a low sodium-low protein diet and when significant polyuria is still present, instruct patients to double voiding to avoid dysfunction and dilatation of the bladder;
- add a thiazide diuretic in children and adults with symptomatic polyuria persisting after a low solute diet. The diuretic dosage should be increased when needed;
- amiloride to be added in cases in which polyuria is still present;
- NSAIDs (indomethacin) are indicated if polyuria persists and there are no contraindications;
- desmopressin might be indicated in patients with persistent polyuria in which NSAIDs are contraindicated or are unresponsive to NSAIDs;

- in children with hereditary NDI frequent water supply and frequent double voiding will prevent dehydration, hypernatremia, and dilatation of the urinary tract and bladder.
- Of note, treatment of patients with low sodium diet, NSAID and thiazide diuretics can increase lithium toxicity and increase the risk of lithium-induced NDI.

## Unconventional Therapeutic Approaches for the Treatment of NDI

### Chemical chaperones

The most prevalent AVPR2 mutations (class II) do not interfere with the intrinsic functionality of receptor, but cause its retention in the endoplasmic reticulum (ER), making it unavailable for AVP binding. The observation of an extensive intracellular retention of a functional AVPR2 suggested the development of small cell-permeable molecules able, either to rescue AVPR2 on plasma membrane (AVPR2 antagonists) or to activate AVPR2 in the ER (AVPR2 agonists).

Rescue of the AVPR2 has been attempted with limited results using chemical chaperones that, in an unspecific way, aid protein folding, such as glycerol and dimethylsulfoxide (DMSO) [51,113].

This strategy based on chemical chaperones was also tested to correct defective AQP2 processing and ER retention in autosomal recessive NDI (Fig. 1C). In CHO and MDCK cells glycerol, trimethylamine N-oxide (TMAO) and DMSO induced redistribution of AQP2 mutants from ER to membrane fraction [78]. Another strategy to enable AQP2 mutants from the ER is the use of heat shock protein 90 (Hsp90) inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG). Hsp90 is a "molecular chaperone" ER-resident/cytoplasmic protein that aids proper folding of proteins and interacts with and promotes ER-associated degradation pathway (ERAD) of several aberrantly folded proteins [113]. 17-AAG partially corrected NDI in conditional AQP2-T126M knock-in mice with partial rescue of defective AQP2-T126M cellular processing [99].

### Nonpeptide AVPR2 antagonists: pharmacological chaperones

Nonpeptide vasopressin receptor antagonists commonly are named vaptans, vap for vasopressin, tan for antagonists [114]. The structure of these compounds imitates the structure of the native hormone AVP, and these antagonists interact with the binding pocket of AVP [115].

Nonpeptide agonists are small cell-permeable molecules that can enter the cell to bind class II mutant AVPR2 in the ER and stabilize their conformation. As a result of their interaction, the receptor mutants are no longer recognized by the ER quality control mechanism as being misfolded, which allows them to exit the ER, achieve mature glycosylation in the Golgi compartment and be inserted into the plasma membrane. Once on the plasma membrane, sufficiently high levels of AVP can displace the antagonist and activate mutant receptor. Similarly to ER-resident molecular chaperones, these cell-permeable antagonists are called pharmacological chaperones [116]. The AVPR2-selective antagonists SR121463 (satavaptan) [117], VPA985 (lixivaptan) [117], OPC41061 (tolpavtan), OPC31260 (mozavaptan) [113] and the AVPR1a antagonist SR49059 (relcovaptan) [118] and the nonselective AVPR1a/AVPR2 antagonist YM087 (conivaptan) [119] promoted adequate maturation and cell surface expression of AVPR2 mutants, with restoration of their ability to initiate a cell response upon AVP binding.

The proof of principle of the usefulness of these molecules has been

provided by a small-scale clinical trial conducted in 2006 by Bernier et al. [119]. This study showed that in five patients who had X-linked NDI and harbored three different AVPR2 mutations, SR49059 had beneficial effects on urine volume and osmolality starting a few hours after administration. Unfortunately, the clinical development of SR49059 has been interrupted during the course of these studies because of a possible interference with the cytochrome P450 metabolic pathway [119].

Moreover YM087 that is in advanced clinical testing phase and has an excellent safety profile for another application [120], was found to rescue cell surface expression and function of nine missense AVPR2 mutant receptors.

Some considerations about antagonists' characteristics, mechanism of action and therefore applicability for different AVPR2 mutants, are important. First, agonist's effects depend on the type and the location of the AVPR2 mutation [116].

Secondly, the extent of plasma membrane rescue of AVPR2 mutants is determined by the affinity of the antagonist but a crucial aspect necessary for functional rescue is displacement of the AVPR2-bound antagonist by AVP in order to generate a cAMP response. Displacement of the antagonist by AVP and subsequent cAMP generation inversely correlated with the antagonist's affinities for the AVPR2. Therefore, the most efficient overall functional rescue depends on a balance between the ability of a compound to promote the mutant receptor's trafficking to the plasma membrane, and its ability to be displaced by a natural or synthetic agonist there [113].

Finally, stimulation of AVPR2 by AVP leads to increased internalization and rapid degradation of receptors by the beta-arrestin-MAPK-pathway [121], counteracting the effect of any potential rescuing molecule.

### Nonpeptide AVPR2 agonists

Similar to cell-permeable antagonists, cell-permeable agonists are able to bind AVPR2 mutants trapped in the endoplasmic reticulum. Rather than stabilizing their conformation, some agonists directly activate these intracellularly-located AVPR2 mutants by signaling to pre-formed receptor-G protein-adenylate cyclase (AC) complexes. The resulting cAMP response will then activate protein kinase A (PKA) to induce trafficking of AQP2 storage vesicles to, and their fusion with the apical membrane, thereby attenuating the NDI phenotype [116].

The pharmaceutical industry has recently made efforts to develop nonpeptide AVPR2-specific agonists for oral administration. Likely, their small-molecule composition and relatively high hydrophobicity allows them to pass cell membranes, thereby facilitating an efficient uptake by the intestinal tract compared to 'classical' peptide-based agonists such as dDAVP [116].

Agonists OPC51803, VA999088 and VA999089, but not vasopressin, activate NDI-causing AVPR2 mutants at their intracellular location, without changing their maturation and at a sufficient level to induce the translocation of aquaporin-2 to the apical membrane, critical step in renal water reabsorption. These compounds showed to be effective in six out of seven AVPR2 mutants (L44P, Y128S, I130F, S167T, Y280C and P322S, but not S167L) [122].

Moreover OPC51803 produced a significant antidiuretic action after single and multiple oral dosing in Brattleboro rats, which have functional AVPR2s but lack AVP, and in normal-hydrates rats [123].

The direct activation of functional ER-retained AVPR2 mutants

observed with these nonpeptide AVPR2 agonists indicates that treatment is likely more advantageous over nonpeptide antagonists because rescue of cell surface expression of the AVPR2 mutants and subsequent displacement of the antagonists by endogenous AVP is not required [122]. In addition proteasome degradation of the ER-trapped receptors is not increased upon intracellular activation by the non-peptide agonists lacking pharmacochaperone function [122]. Another advantage of the use of nonpeptide agonists, as well as antagonists, for intracellular AVPR2 stimulation is the high selectivity of non-peptide compounds for the AVPR2.

This should prove to minimize the side-effects of administration of those therapeutics, since no other cellular mechanisms are expected to be activated [41].

Jean-Alphonse et al. [124] identified nonpeptide agonists for AVPR2 that also acted as pharmacochaperones: MCF14, MCF18 and MCF57. These compounds promoted maturation and membrane rescue of L44P, A294P, and R337X AVPR2 mutants and restored a functional AVP-dependent cAMP signal. Contrary to pharmacochaperone antagonists, MCFs directly activate cAMP signaling once the complex MCF-AVPR2 is at the cell surface and agonist's functional rescue is not a subtle balance between the ability of the ligand to target cell surface expression of the AVPR2 and its possibility to be displaced by AVP for receptor activation. In addition, these molecules displayed original functionally selective properties (biased agonism) toward the AVPR2, being unable to recruit arrestin, trigger receptor internalization, or stimulate mitogen-activated protein kinases, contrary to the hormone AVP [124].

In conclusion, the effect of nonpeptide agonists on AVPR2 mutants is mutation- and agonist-dependent, and studies clearly demonstrate the potential of cell-permeable AVPR2 agonists as future therapies for NDI resulting by misfolded AVPR2 mutants [116].

### Bypassing AVPR2 signaling

Many strategies have been proposed to bypass the defective AVPR2 signaling and restore physiological AQP2 trafficking and/or expression in collecting duct principal cells of NDI patients. Owing to the lack of AVPR2-induced cAMP signaling, possible approaches to restore proper AQP2 transcription and/or translocation of storage vesicles to the apical plasma membrane are:

- activation of other G proteins-coupled receptors (GPCRs) linked to Gs/adenylate cyclase expressed in collecting duct (CD) principal cells;
- activation of cAMP-independent signaling cascades. This approach is based on the fact that AQP2 insertion on the plasma membrane of CD principal cells could also be accomplished by elevation of intracellular cGMP;
- use of statins to accumulate AQP2 at the apical plasma membrane.

**Cyclic AMP pathway activation:** Several studies suggest that physiological (secretin, calcitonin) or synthetic (ONO) agonists of other GPCRs expressed in CD principal cells also exhibit anti-diuretic functions [125-127].

**Secretin-** The secretin receptor (SCTR) is a GPCR known to interact to both Gs and Gq, with the stimulatory cAMP response being most prominent and sensitive [128]. Of note, SCTR-null mice display mild polyuria, polydipsia, distension of the renal pelvis and reduced renal expression of AQP2 [125]. The same authors showed



that SCTR is expressed in the kidney medulla [125], in agreement with previously published data [129]. Our recent study [130] showed, for the first time, that the SCTR is expressed at the basolateral membrane of AQP2-expressing CD principal cells in mice and humans, prompting us to analyze the effect of SCT *ex vivo* on kidney slices and *in vivo* in AVPR2-KO mice (X-NDI mice [106]). We provided compelling evidence that SCT induces a dose-dependent rise in intracellular cAMP concentrations in CD tubule suspensions and promotes AQP2 apical expression in freshly isolated kidney slices from control and X-NDI mice. In addition, chronic infusion of SCT increases AQP2 abundance but not its apical expression in X-NDI mice. Of note, in SCT-infused X-NDI mice, a single injection of fluvastatin (see below), a drug that induces AQP2 membrane accumulation in wt C57BL/6 mice [131], promotes AQP2 membrane expression and greatly improves the urine concentration ability [130].

**Calcitonin-** Previous works with calcitonin (CT) showed a possible vasopressin-like effect on electrolyte and renal water reabsorption [126,127]. Mainly secreted by thyroid parafollicular cells [132], calcitonin binds to two different class of GPCRs (CT<sub>(A)</sub> and CT<sub>(B)</sub>) which are both associated with the G-protein Gs/adenylyl cyclase/cAMP pathway [133-135]. Renal distribution of CT receptors differs among species [136,137]. In human, CT stimulates adenylyl cyclase activity in thick ascending limbs and in cortical and medullary collecting ducts [138] while in rat, which express only CT<sub>(A)</sub>, binding sites were detected in the cortical collecting ducts, distal convoluted tubules and in thick ascending limbs [137-140]. Overall, the similar localization of both CT and AVPR2 receptors in some of the same tubule segments is consistent with the observation that CT might exert an AVP-like effect on water reabsorption [126]. More recently, Bouley and colleagues investigated the effect of CT on AQP2 trafficking *in vitro*, *ex vivo*, and *in vivo* using LLC-PK1 kidney epithelial cells, kidney slices, and CT-infused AVP-deficient Brattleboro rats, respectively [141]. CT induced an increase of intracellular cAMP in AQP2-expressing LLC-PK1 cells that express endogenous CT receptor, resulting in AQP2 membrane accumulation. In addition, immunocytochemistry on Brattleboro rat kidneys showed a CT-induced increase of AQP2 membrane accumulation in cells from cortical collecting ducts, in parallel with a significant but transient reduction in urine volume and a two fold increase in urine osmolality when compared with control rat. However, since the amount of AQP2 protein was not significantly changed after 24-hour infusion of CT, a combination of agents that stimulate both AQP2 expression and apical accumulation in both cortical and medullary collecting duct is mandatory to elicit a robust antidiuretic response in X-NDI.

**Prostaglandin receptors activation:** ONO- ONO-AE1-329 (ONO) is a selective agonist of the EP4 PGE2 prostanoid receptor [142], a Gs-coupled receptor, expressed at significant levels in mouse and rat inner medullary collecting duct (IMCD) cells [106,143]. In a recent paper Li and colleagues have shown that both acute and chronic treatment of AVPR2 mutant mice with ONO greatly reduced all major manifestations of XNDI (e.g. large dilatations of the renal pelvic space, kidney failure secondary to bilateral hydronephrosis and reduced glomerular filtration rate), leading to striking reductions in urine output and water intake and pronounced increases in urine osmolality [106]. In addition, prolonged treatment of AVPR2-KO mice with ONO significantly increased renal AQP2 levels probably due to EP4 receptor-mediated elevations of cAMP levels in kidney collecting duct cells. In fact, ONO treatment of collecting duct tubule preparations of AVPR2-KO mice led to a pronounced increase in cAMP levels and enhanced water permeability. Other agonists specific for EP2 (butaprost) and EP4 (CAY10580) were shown to increase AQP2 trafficking [144], although

the mechanisms of action are likely to be different, because only EP2 stimulation increased cAMP in MDCK cells [144]. In the same study, butaprost was able to reduce urine volume and increase urine osmolality by up to 65% in a rat model of X-NDI. Overall, selective EP4/EP2 receptor agonists may represent a new class of drugs useful for the treatment of XNDI due to their direct action on collecting duct water permeability.

However, it is important to note that intrarenal PGE2 infusion promotes diuresis [145], probably via activation of other PGE2 receptor subtypes mediating inhibition of salt and water absorption along the nephron [146]. Inhibition of these latter PGE2 effects by indomethacin, which reduces tissue PGE2 levels *via* nonselective inhibition of cyclooxygenase 1 and 2, is thought to contribute to the ability of this drug to reduce urine production in XNDI patients [147]. Therefore, selective PGE receptor antagonism may represent an efficient means of controlling water excretion and that every effort should be made to develop other PGE receptor inhibitors that target other PGE receptor isoforms such as EP3, which is found to be expressed in the collecting duct [148].

Finally, another potential strategy to increase cytosolic cAMP level is inhibition of phosphodiesterases (PDE). A recent study showed that rolipram, a PDE4 inhibitor, increases urine osmolality in a hypercalcemia-induced NDI mice model [83]. Accordingly with the known action of this PDE inhibitor, rolipram increases cAMP content in the papillae, AQP2 phosphorylation, and AQP2 apical translocation. Although PDE3 and PDE5 are thought to be present in the collecting ducts [149], inhibitors of these PDEs were ineffective. In a clinical study, however, Bichet and colleagues had not found any improvement of polyuria in two NDI patients treated with rolipram [150], suggesting that inhibition of PDE4 is still not an option for the treatment of all NDI patients.

**Cyclic GMP (cGMP) pathway activation:** A potential pharmacological therapy for X-NDI is to bypass AVPR2 and cAMP activation pathway *via* the activation of the cGMP pathway. The activation of a cAMP-independent and cGMP-dependent pathway for AQP2 membrane insertion by different cyclic guanosine monophosphate (cGMP) pathway activators has been previously proposed [151-153]. Nitric oxide (NO) donors, such as sodium nitroprusside (SNP) and NONOate, as well as the nitric oxide synthase (NOS) substrate L-arginine, induced AQP2 translocation from intracellular vesicles to the apical membrane by increasing cGMP levels in rat kidney slices and AQP2-transfected LLC-PK1 cells. In addition, atrial natriuretic peptide (ANP), which increases cGMP levels by activating membrane-bound guanylyl cyclase, stimulates AQP2 membrane insertion in the principal cell of ANP infused rats [153]. The mechanism by which cGMP induces AQP2 trafficking is still puzzling. On one hand, it has been shown that purified AQP2 COOH tail can be phosphorylated by PKG [151]. On the other hand, neither the possibility that PKG phosphorylates PKA nor that cGMP activates directly PKA can be reasonably eliminated.

In the absence of non-functional AVPR2, however, AQP2 expression levels are also reduced, because cAMP, generated through the activation of the AVPR2, stimulates AQP2 transcription through a cAMP-responsive element in its promoter [154-157]. However, increased AQP2 expression following the activation of the cGMP pathway is still under discussion. In the mouse cortical collecting duct cell line (mpkCCD), vasopressin increases endogenous AQP2 expression [158]. Therefore, Boone and colleagues used this cell line to determine if the activation of the cGMP-signaling pathway not only

induces AQP2 translocation, but also increases AQP2 expression. These authors found that ANP, L-arginine and 8-Br-cGMP induce translocation of AQP2 while do not significantly affect AQP2 expression in mpkCCD cells [159]. Taken together these data suggest that the beneficial effect of compounds activating the cGMP pathway compounds to relieve NDI may be improved when combined with agents that stimulate AQP2 expression.

Elevation of cGMP could also be achieved by cGMP phosphodiesterases inhibitors. Several isoforms are expressed along the nephron, such as cGMP-sensitive PDE (PDE 5), and cAMP/cGMP selective PDE (PDE 1) [147]. Acute exposure of both LLC-PK1 cells stably transfected with AQP2 (LLC-AQP2 cells) and collecting duct principal cells in tissue slices of rat kidney to sildenafil citrate and 4-[[3',4'-methylene-dioxybenzyl]amino]-6-methoxyquinazoline (MBMQ), both of which are PDE5 inhibitors, leads to a vasopressin-like stimulation of AQP2 accumulation in the plasma membrane. In addition, acute exposure to sildenafil causes accumulation of AQP2 in the apical plasma membrane domain of collecting duct principal cells in AVP-defective Brattleboro rats [152]. In addition, treatment with sildenafil citrate increases both cGMP levels in medullary collecting duct cell suspension and AQP2 expression in crude membrane fractions of Wistar rats with lithium-induced NDI. Therefore, sildenafil ameliorates experimental Li-induced NDI progression by reducing polyuria and increasing urinary osmolality [160]. Overall, pharmacologically mediated PDE5 inhibition can potentially be used to bypass the AVPR2 signaling cascade, leading to AQP2 appearance on the plasma membrane of epithelial cells.

**Statins:** Many recent papers have highlighted a new pleiotropic effect of statins in promoting AVP-independent apical localization of AQP2 in renal cells *in vitro* and *in vivo* [130,131,161,162]s. Currently, six different statins (simvastatin, pravastatin, lovastatin, fluvastatin, atorvastatin, and rosuvastatin) are approved for the treatment of hypercholesterolemia in humans. All statins block the conversion of HMG-CoA to mevalonic acid with consecutive attenuation of the biosynthesis of cholesterol [163], greatly reducing the primary and secondary cardiovascular risk. Many of the so-called pleiotropic effects of statins have been shown to be secondary to the inhibition of the synthesis of isoprenoid intermediates of the mevalonate pathway including farnesyl pyrophosphate and geranylgeranyl pyrophosphate [164]. Isoprenoids are important substrates for the post-translational modification of many signaling proteins including small GTP-binding proteins. These latter proteins play crucial roles in many cellular functions including cytoskeletal assembly and protein and lipid trafficking [165]. Independent results show that in statins-treated renal cells, reduced isoprenylation of RhoA leads to actin cytoskeleton depolymerization and AQP2 accumulation at the plasma membrane [131,162]. *In vivo*, treatment of AVP-deficient Brattleboro rats [166] and conditional mice model of X-linked NDI [106] increased AQP2 expression at the apical plasma membrane and induced a marked reduction of the polyuria and a consistent increase of urine osmolality [131,162,167].

Our group is currently investigating the effect of statins treatment on AQP2 intracellular trafficking in humans. Based on these observations, it has been proposed that statins may improve the beneficial effects of the current therapy and further reduce the polyuria of NDI patients with a positive impact on their quality of life.

### Gene therapy

As a recessive genetic disease, NDI could theoretically be cured

by replacing the defective AQP2 or AVPR2 genes, i.e. gene therapy. This exciting prospect would eliminate lifelong, the often inadequate management with thiazide diuretics. The many obstacles to gene therapy for NDI may be surmountable with a coordinated multidisciplinary approach. Preliminary studies demonstrated successful transgene expression in tubular epithelial cells, specifically in the S<sub>3</sub> segment of the proximal tubule and intercalated cells, after intrarenal administration of a recombinant adeno-associated viral (rAAV) vector and provided the impetus for further studies to exploit its use as a tool for gene therapy in the kidney [168,169]. Recombinant adeno-associated viral vectors have several distinct advantages over other gene delivery vectors because rAAV results in long-term transgene expression and infects cells with no significant side effects, particularly with respect to immune responses [170]. Gene therapy may eventually become applicable to the congenital forms of NDI. At present all gene-therapeutic approaches lack safety and efficiency, which is of particular relevance in a disease that is treatable by an adequate water intake. Therefore, it is difficult to predict when or even if this treatment will become a reality.

### Concluding Remarks

Hereditary nephrogenic diabetes insipidus is a relatively rare disorder characterized by unresponsiveness of the kidney to the antidiuretic action of vasopressin. In the hereditary forms it results from genetic abnormality in the key component of the kidney water retaining machinery: AVPR2 and AQP2. Early diagnosis and prompt therapy in newborns prevent mental retardation and permit children to survive into adulthood. Unfortunately, the current therapeutic options are limited and only partially beneficial.

The most common defect are mutations of the AVPR2 leading to X-linked NDI and only a small number of patients carry genetic defects in the AQP2 gene and show autosomal recessive NDI.

Therefore, the aim of the current research is the identification of pharmacological chaperones to restore routing of partially functional proteins to the plasma membrane. In X-linked NDI research is also focused on membrane-permeable agonists that could stimulate AVPR2 within the cell and elicit a cAMP-mediated response.

More recently, novel approaches have been proposed as potential treatment of X-linked NDI to bypass the AVPR2 defect: activation of other GPCRs, activation of the cGMP pathway, and administration of statins.

For autosomal dominant NDI, misrouting of AQP2 or defects in the AQP2 phosphorylation sites can explain the lack of AVP effect. Different approaches need to be identified to cure this particular form of NDI. Gene therapy might represent the only "real cure" for this defect. Unfortunately few and very preliminary attempts have been made toward the establishment of a gene therapy approaches to cure NDI. Further investigation *in vitro* or using the available animal models of the disease, combined with clinical trials, will eventually lead to identify one or more strategies that will improve or replace the current conventional therapy and grant NDI patients a better quality of life.

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